Chapter 14

Methods to Assess Intestinal Stem Cell Activity in Response to Microbes in *Drosophila melanogaster*

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Abstract

Drosophila melanogaster presents itself as a powerful model for studying the somatic stem cells of the gut and how bacteria affect intestinal homeostasis. The *Gal4/UAS/Gal80^{ts}* system allows for temporally controlled expression of fluorescent proteins, RNAi knock-down, and other genetic constructs targeted to specific cell populations in the midgut. Similarly, FLP/FRT-mediated somatic recombinations in intestinal stem cells (ISCs) are utilized to visualize and analyze the clonal lineages of individual or populations of stem cells. Live imaging microscopy and immunofluorescence allow both qualitative and quantitative characterization of stem cell shape, proliferation, and differentiation. Here, we detail the use of these tools and techniques for studying gut performance during and following a bacterial infection in the adult fruit fly.

Key words *Drosophila*, Intestinal stem cell, Epithelium renewal, Bacterial infection, Midgut homeostasis, Lineage analysis, Immunostaining

1 Introduction

The gut of *Drosophila melanogaster* is composed of a monolayer of epithelial cells, surrounded by two layers of visceral muscles and arranged into a tube with three distinct compartments: the foregut, the midgut, and the hindgut (Fig. 1) [1, 2]. The foregut and hindgut are derived from the ectoderm and their epithelium is covered by chitin, while the midgut is derived from the endoderm, covered by a chitinous matrix (the peritrophic matrix), and serves as the primary site of nutrient processing and absorption [1]. Three types of cells compose the epithelia of the midgut: large, nutrient absorbing enterocytes (ECs), small, secretory enteroendocrine (EE) cells, and pluripotent intestinal stem cells (ISCs).

ISCs in *Drosophila*, like those in mammals, maintain the gut by self-renewing division, yielding one new ISC and one nondividing progenitor cell called an enteroblast (EB) [3, 4]. EB cells undergo further fate decision and ultimately differentiate to become new ECs or EE cells, replacing the old intestinal cell population [5].

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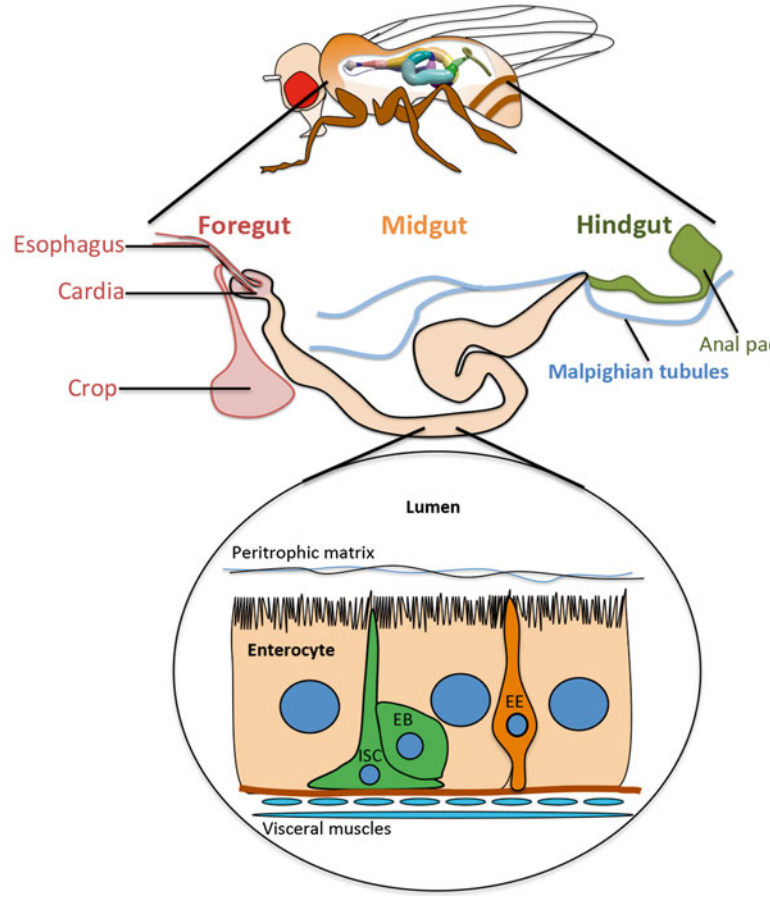

Fig. 1 The *Drosophila* gut. The gut of an adult fruit fly is organized into three distinctive regions: the foregut, the midgut, and the hindgut. The foregut comprises the esophagus and the crop, which acts as a storage organ and initiates nutrient processing. Food is then passed on to the midgut where the majority of nutrient digestion and absorption occurs. Finally, the hindgut functions to reabsorb water from waste material before its removal. The midgut epithelium is surrounded by visceral muscles and is composed of four primary cell types: Enterocytes (ECs), Enteroendocrine (EE) cells, Intestinal Stem Cells (ISCs), and Enteroblasts (EBs). ECs and EEs are differentiated and carry out absorptive and neurosecretory functions, respectively. ISCs replenish old or destroyed cells through self-renewing division, yielding a new ISC and an EB, which is dedicated to differentiate into either an EC or an EE cell

Complete turnover of the midgut is accomplished by this process in 10–15 days under basal conditions, but is greatly accelerated in response to intestinal damage and microbial pathogens [2, 6, 7]. The discovery of ISCs in the midgut of *Drosophila*, and the wealth of genetic tools established in the fruit fly, make it an ideal and exciting model for studying the behavior of ISCs during infection.

In this chapter, we describe techniques for performing oral infections in *Drosophila* and monitoring ISC proliferation and

subsequent lineage. The *Gal4/UAS/Gal80^{ts}* system allows for fluorescence and lacZ labeling of particular intestinal cell types by making expression of a reporter gene dependent upon the expression of cell-specific enhancers. In addition, immunostaining allows cell types to be labeled according to cell-specific, targetable antigens. Furthermore, visualization of progenitor lineages, stem cell division rates, and global tissue renewal can be accomplished with diverse genetic systems such as *esg-Gal4^{ts}*, *tub-FRT-lacZ clones*, *esg$^{F/O}$*, *MARCM*, and *Twin-spot MARCM* (*see* Subheading 2.3).

2 Materials

2.1 Fly Rearing and Husbandry

1. *Drosophila* diet: 50 g baker's yeast, 40 g sucrose, 60 g cornmeal, 7 g agar, 16 mL Moldex (10 %), 8 mL acid mix (*see* **Notes 1** and **2**), 1,000 mL deionized water.
2. Standard fly vials (~22 mm in diameter) with *Drosophila* diet.
3. Facilities to maintain flies at 18 and 29 °C.

2.2 Bacterial Cultures

1. Sterile Luria Bertani Broth (LB).
2. LB agar plates: 1.5 % agar in LB, poured into sterile culture plates.
3. Sterile, disposable inoculation loops.
4. Autoclaved Erlenmeyer flasks.
5. Pathogenic bacteria stocks: *Erwinia carotovora* subsp. *carotovora* 15 (*Ecc15*), *Pseudomonas entomophila*, *Serratia marcescens* str. Db11, or *Pseudomonas aeruginosa* (*see* **Note 3**).
6. Shaking incubator thermostated at 29 °C.

2.3 Fly Genetics

1. *Gal4/UAS/Gal80^{ts}* system (Fig. 2a): Allows labeling of specific cells in the gut (*see* **Note 4**). We can induce the *Gal4/UAS* system to visualize different cell populations in the gut by expressing fluorescent proteins in specific cell types. For instance, the *esg-Gal4^{ts}* system (*esg-Gal4*, *Gal80^{ts} UAS-GFP* flies) allows for visualization of progenitors to monitor ISC shape, number, and proliferation [4, 6].
2. *tub-FRT-lacZ* clones: Randomly labels individual stem cells and their progeny in a heat shock-inducible manner (*see* **Note 5**) [8]. Used to study ISC proliferation and ISC lineage.
3. *esg$^{F/O}$* system: Systematically labels all ISCs and their progeny with GFP in an inducible manner (Fig. 2b) [7]. Used to study proliferation of progenitors, ISC lineage, and tissue renewal over time (*see* **Note 6**).
4. MARCM (Mosaic Analysis with a Repressible Cell Marker) clones: Randomly labels individual stem cells and the progeny of one of the two daughter cells [9, 10]. For its application to ISC lineage, see Singh et al. [11].

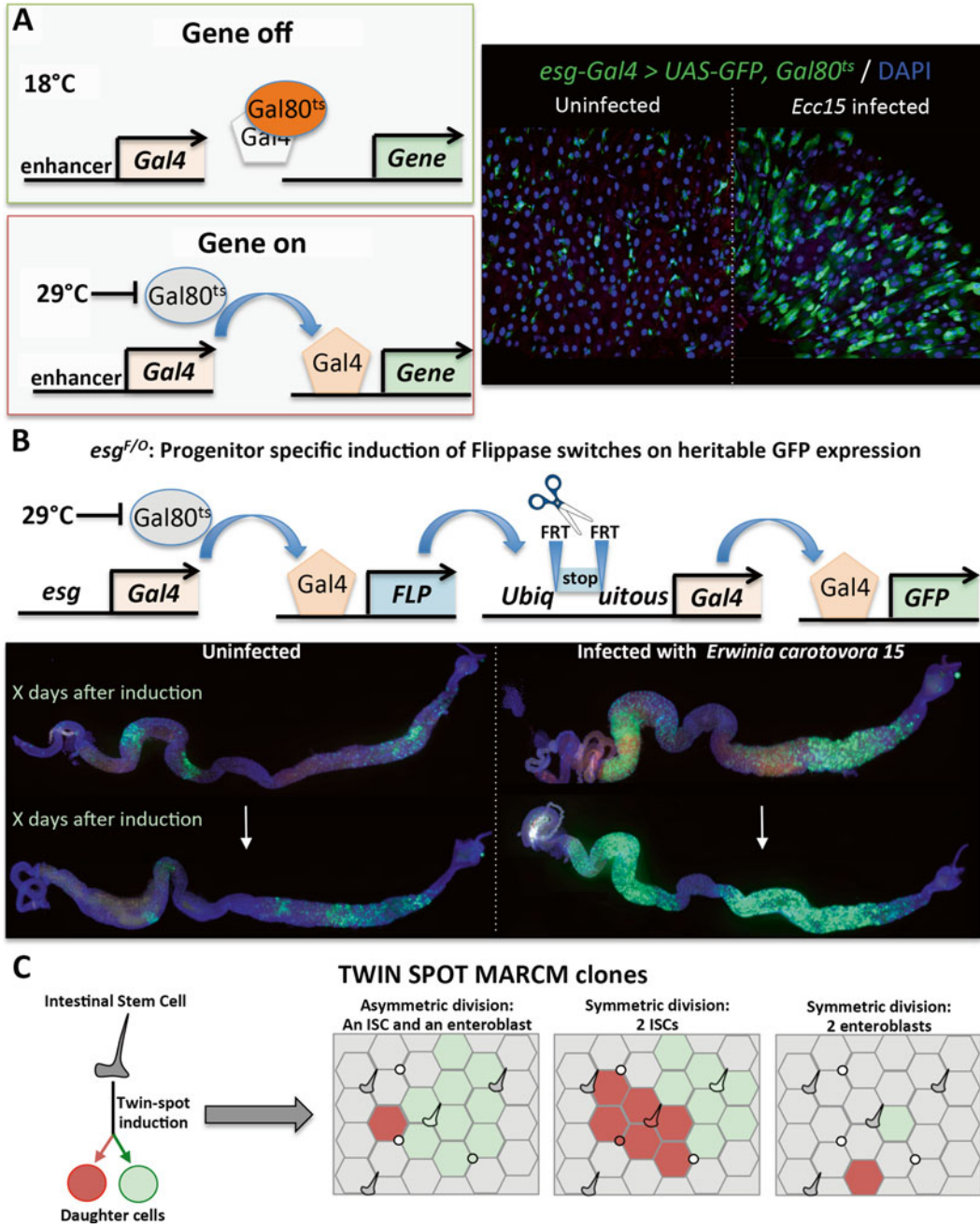

Fig. 2 Fly genetic tools for studying intestinal stem cell activity. (**a**) *Left*: The *Gal4/UAS/Gal80ts* system allows the expression of *UAS-GFP*, and other *UAS*-regulated transgenes, to be induced by Gal4 in a temperature-dependent manner in only the cells where the promoter of the *Gal4* transgene is active. Gal80ts inhibits Gal4 at 18 °C, preventing GFP expression controlled by *UAS*, but becomes inactivated at 29 °C. *Right*: Choosing a promoter expressed in progenitors (*esg*) allows us to visualize the activation of progenitor cells during infection, as illustrated by the diffuse GFP signal in infected guts (*right panel*). (**b**) The *esgF/O* system drives the temperature-dependent expression of FLP recombinase in progenitors (when moved to 29 °C). This triggers the FLP out of the CD2 cassette and the activation of the *act-Gal4* ubiquitous driver in subsequent ISC progeny that therefore expresses GFP. The proportion of GFP positive cells in the midgut reflects turnover rates. Infection with *Ecc15* induces an acceleration of epithelium renewal (see microscopy examples in *lower panel*). (**c**) The *Twin-spot MARCM* system induces the expression of different heritable markers (GFP and RFP) in the two daughter cells of an ISC. This allows establishment of the symmetrical or asymmetrical behavior of ISC divisions, discernible through the analysis of the subsequent lineage of the two daughter cells

5. Twin-spot MARCM system: Randomly labels dividing ISCs and progeny in a heat shock-inducible manner [12, 13]. After mitosis of the parent cell, the two daughter cells are tagged with a different fluorescent reporter. This allows observing the fate of the two daughter cells of an ISC division, monitoring both proliferation and the proportion of symmetric versus asymmetric division (*see* **Note 7**) (Fig. 2c).
6. Additional molecular markers to study ISC in the gut of *Drosophila* can be found in Singh et al. [11].
7. 37 °C water bath.

2.4 Oral Infection

1. Absorbent pads (e.g., Whatman filter paper), cut to the diameter of the fly vials (usually 22 mm).
2. Empty fly vials.
3. Fly vials with *Drosophila* diet.
4. Concentrated sucrose solution in sterilized water (*see* **Note 8**).
5. Bacterial pellet: The infectious dose varies for different bacteria species (i.e., *Ecc15* pellet should have an $OD_{600} = 200$).

2.5 Gut Dissection

1. Multi-well glass dish.
2. Source of CO_2 for anesthetization (Fig. 3a).
3. Forceps (*x*2).

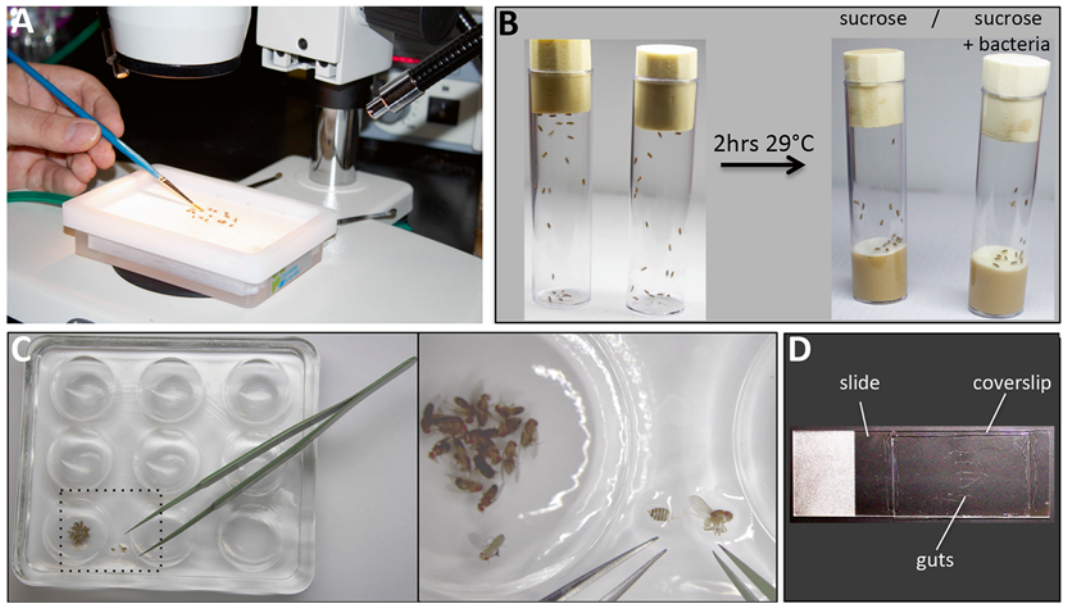

Fig. 3 Steps in studying intestinal stem cells using *Drosophila*. (**a**) Fly pushing and sorting on a CO_2 pad to obtain the desired genotype/phenotype. (**b**) Flies are starved at 29 °C for 2 h in empty tubes before being transferred onto filter pads with sucrose (control) or bacteria and sucrose mixes. (**c**) Dissection of midguts from anesthetized flies in PBS, on a spot plate. (**d**) Guts positioned on a slide with mounting solution under a coverslip

4. 70 % EtOH.
5. Sterile 1× PBS (Phosphate Buffer Saline).

2.6 Live Imaging

1. Sterile, 1.5 or 2 mL centrifuge tubes.
2. PBT solution (0.05 % Tween 20): Add 25 μL of Tween 20–50 mL 1× PBS and mix.
3. DAPI staining solution: Add 1 μL of 20 mg/mL DAPI dilactate in sterile water to 50 mL PBT. Store at 4 °C.
4. PBS/glycerol (1:1) or antifadent mounting medium (Citifluor AF1 or Vectashield).
5. Glass microscope slides.
6. Glass coverslips.
7. Nail polish (to seal coverslips on slides).

2.7 Immunostaining

1. Sterile, 1.5 or 2 mL centrifuge tubes.
2. PBT solution (0.1 % Tween 20): Add 50 μL of Tween 20–50 mL 1× PBS and mix.
3. 4 % paraformaldehyde (PFA) fixative in PBT (0.1 % Tween 20). Store solution at −20 °C or at 4 °C not more than 2 days.
4. PBTA: PBT solution (0.1 % Tween 20) with 1 % bovine serum albumin (BSA).
5. Primary antibody stocks: mouse anti-GFP (Roche), mouse anti-RFP (Clontech), rabbit anti-PH3 (Millipore) for cells undergoing mitosis, mouse anti-Prospero (Developmental Studies Hybridoma Bank) for EE cells, anti-PDM1 for ECs [14]. Additional primary antibodies used to study ISC in the gut of *Drosophila* can be found in Singh et al. [11].
6. Secondary antibody stocks: Alexa-488 anti-mouse (Invitrogen), Alexa-594 anti-rabbit (Invitrogen).
7. PBS/glycerol (1:1) or antifadent mounting medium (Citifluor AF1 or Vectashield).
8. Glass microscope slides.
9. Glass coverslips.
10. Nail polish (to seal coverslips on slides).

3 Methods

3.1 Fly Rearing and Husbandry

1. *Drosophila* stocks: Maintain flies by transferring adults to new vials every 2–3 days at room temperature or in a 25 °C incubator, or every 7 days in an 18 °C incubator (stocks with a Gal80^{ts} system). Maintain at a ~12/12 h light/dark cycle.

3.2 Bacterial Cultures

1. Pour 500 mL of sterile LB medium into a sterile, autoclaved Erlenmeyer flask with foil cover (*see* **Note 9**).
2. Locate a single colony on an LB bacterial stock plate and gently scrape it onto a sterile, disposable inoculation loop.
3. Transfer the colony to the Erlenmeyer flask with LB medium and seal the flask with a sterile cover (for aerobic bacteria, it should not be air tight).
4. Secure the Erlenmeyer flask in a shaking incubator at 29 or 37 °C, depending on the growth requirements of the bacteria, and incubate for 16 h while shaking at 200 rpm (*see* **Note 10**) to reach stationary growth phase.
5. Pour the liquid culture into a sterile centrifuge flask and centrifuge at 4 °C and 3,220 × *g* for 15 min.
6. Empty most of the LB medium from the centrifuge flask.
7. Use a pipette and sterile tips to resuspend the bacterial pellet in the remaining LB medium. Transfer the liquid, concentrated bacterial pellet into a sterile 15 mL tube.
8. Make a 1:1,000 dilution of the pellet in sterile water in a separate test tube. Measure the OD_{600} absorbance of the dilution and subsequently calculate the concentration of the bacterial pellet. Adjust the bacterial concentration ($OD_{600} = 200$ for *Ecc15*).
9. Store bacterial pellet at 4 °C for up to 1 week.

3.3 Fly Genetics

1. Raise Gal80^{ts} stocks (*esg-Gal4^{ts}* and *esg$^{F/O}$*) at 18 °C and shift to 29 °C 2 days prior to infection for activation of Gal4-mediated expression.
2. For flies using FLP/FRT-mediated recombination and *hsFLP* (*tub-FRT-lacZ*, *MARCM*, and *Twin-spot MARCM* systems), cross stocks appropriately for the F1 progeny to carry all required transgenes [8, 11, 12]. Raise the F1 progenies at 18 °C, then heat shock for 40 min at 38 °C, and use 2 days post-clonal induction.
3. Sort flies of the proper genotype on a CO_2 pad prior to infection (Fig. 3a).

3.4 Oral Infection

1. Flip experimental flies into empty fly tubes and put at 29 °C for 2 h (*see* **Note 11**).
2. Prepare 2.5 % and 5 % sucrose dilutions in sterile water. Mix the 5 % sucrose solution with an equal volume of the bacterial pellet (at $OD_{600} = 200$ for *Ecc15*) to create the infection mix.
3. Set up labeled standard fly tubes with diet. Place an absorbent pad into a tube and push it down until it contacts the diet.

The pad should completely cover the diet. Immediately add 150 μL of either 2.5 % sucrose, for controls, or sucrose and bacteria mix, for infections. Repeat for all tubes.

4. Flip flies into appropriate tubes for infection and controls (sucrose). Incubate flies at proper infection temperature for required infection time (*see* **Note 12**).

3.5 Gut Dissection

1. Prepare a clean multi-well glass spot plate and place under a dissection scope (*see* **Note 13**).
2. Anesthetize flies using CO_2 source.
3. Transfer flies to a spot plate well containing 70 % ethanol and briefly submerge (*see* **Note 14**).
4. Remove ethanol and replace with 1× PBS.
5. Use forceps to create a drop of PBS on a flat portion of the spot plate and transfer a fly into the droplet. There should be enough PBS covering the fly that the gut remains submerged during dissection.
6. Using two pairs of forceps, decapitate the fly with a clean stroke across the "neck." Ensure that the esophagus is completely severed.
7. Carefully separate the thorax from the abdomen by bracing one pair of forceps against the thorax while using the other to hold the first abdominal segment, and pull it away from the thorax. Stop once the two are separated and the gut is visible between them.
8. Sever the last two abdominal segments by pinning the end of the abdomen down with one pair of forceps and slicing across it with the other. Carefully pull the remainder of the abdomen away from the thorax and off of the gut. If the gut remains attached, either in the thorax or the abdomen, locate the crop and use it to gently pull the gut away from these segments.
9. Use the forceps to puncture the crop without removing it (*see* **Note 15**).
10. Store the gut in 1× PBS and proceed to Subheadings 3.6 or 3.7.

3.6 Live Imaging

1. Dissect guts for live imaging (*see* Subheading 3.5).
2. Transfer 3–6 guts into a 1.5 mL centrifuge tube with 0.5–1 mL of DAPI staining solution. Incubate guts at room temperature for 10–15 min.
3. Rinse three times with 1× PBS. First and second washes are quick (1 min). The last wash is 5 min.
4. Mount guts on microscope slides in mounting solution or PBS/glycerol.

5. Carefully lay a coverslip over the sample in mounting solution. Carefully remove any excess that oozes from between the slide and coverslip with a Kimwipe.
6. Seal the slide with nail polish.
7. Analyze guts using fluorescent or laser confocal microscopy (Fig. 3d).

3.7 Immunostaining

1. Dissect guts to be stained (*see* Subheading 3.5).
2. Transfer 3–6 guts into a 1.5 mL centrifuge tube with 1 mL of 4 % PFA fixative in PBT. Fix guts at room temperature for 30 min in PFA/PBT.
3. Wash 2–3 times in PBT. First and second washes are quick (1 min). Last wash is 5–10 min.
4. Tissues may be stored in the dark and at 4 °C at this stage before continuing with staining, but only for 1–2 days.
5. Block the epitopes by incubation with PBTA for 1 h.
6. Remove PBTA and incubate in primary antibody hybridization solution overnight in the dark at 4 °C. The hybridization solution is made by diluting the antibody to the proper concentration in PBTA (*see* **Note 16**).
7. Rinse in PBTA, three times 10 min each.
8. Incubate guts with the secondary antibody in PBTA and counterstain. Typical nucleus counterstain is DAPI or TO-PRO-3 (Invitrogen). Depending on the type of staining, this step may occur from 2 h of incubation to a new overnight treatment.
9. Wash three times in PBT, 10–30 min each.
10. Mount and image guts (*see* Subheading 3.6, **steps 4–6**).

4 Notes

1. Acid mix is made by combining a solution of 8.3 mL phosphoric acid in 91.7 mL dH_2O and a solution of 83.6 mL propionic acid in 16.4 mL dH_2O.
2. Mix yeast, sucrose, cornmeal, and agar into the water and autoclave on a liquid cycle to dissolve. Add Moldex and acid mix to the diet once it is cool enough to handle with bare hands. Dispense the diet rapidly into empty fly tubes.
3. *Ecc15* is used to induce nonlethal oral infections, in which flies are able to repair and recover from damage [6, 15]. Oral infections with *P. entomophila* are nonlethal at low doses but ultimately lethal at high doses and associated with high levels of Reactive Oxygen Species (ROS) and pore-forming bacterial toxins [15–18]. Oral infections with *S. marcescens* are lethal

due to the ability of the bacteria to cross the epithelial barrier of the gut and establish a systemic infection [19–21]. *Pseudomonas aeruginosa* induces cell death in the gut and promotes ISC proliferation [22].

4. The basis of the *Gal4/UAS* transgenic system is the generation of transgenic flies that bear either Gal4 expressing transgenes that express the Gal4 yeast transcription factor in a cell-specific manner (dependent of the promoter cloned in front of *Gal4*) or inducible transgenes that are controlled by Gal4 target sites: Upstream Activation Sequence (*UAS*) enhancers. The *UAS* transgene can induce the expression of a fluorescent reporter such as *GFP* or *RFP*. Flies with *UAS* and *Gal4* transgenes are crossed together and, in the F1 progeny, the *UAS* transgene is bound and transactivated by Gal4 only in cells with active *Gal4* expression. This system is further implemented by incorporation of $Gal80^{ts}$, encoding a thermosensitive form of Gal80, which acts as a Gal4 antagonist. The addition of ubiquitously expressed $Gal80^{ts}$ to the *Gal4/UAS* constructs allows the expression of the transgene in flies to be induced by incubation at 29 °C, a temperature at which $Gal80^{ts}$ is inactivated (Fig. 2a). The promoter of *delta* is used to drive expression in ISCs (*delta-Gal4*), the promoter of *Su(H)* is used for EBs (*Su(H)-Gal4*), *Myo1A* for ECs (*Myo1A-Gal4*) [23], *prospero* for EE cells (*prospero-Gal4*), and *escargot* for expression in both ISCs and EBs (*esg-Gal4*) [4].
5. The *tub-FRT-lacZ* clone system makes use of a heat shock-induced, FLP recombinase-dependent, chromosome recombination that results in a heritable expression of *tub-lacZ* in the progeny of a cell [8]. Two homologous chromosomes bear FRT sites (FLP recombination targets), one containing the ubiquitous promoter of *tubulin* (*tub-FRT*) and one containing the gene encoding β-galactosidase (*FRT-lacZ*). The two stocks are crossed and the F1 progeny is collected. Upon heat shock at 38 °C, *hsFLP* is expressed in the F1 progeny, triggering *FRT*-mediated recombination and reactivation of *lacZ* expression by joining the *tubulin* promoter to *lacZ* (*tub-lacZ*), thereby inducing *lacZ* expression in all daughter cells.
6. The $esg^{F/O}$ system (*esg-Gal4*, $Gal80^{ts}$, *UAS-FLP*, *act>CD2>Gal4*, *UAS-GFP*) uses a temperature-sensitive inducible *Gal4*, driven in progenitor cells (*esg-Gal4*, $Gal80^{ts}$) to express FLP recombinase (*UAS-FLP*) in all intestinal progenitors. In this line, FLP removes an FRT-flanked CD2 cassette, allowing *Gal4* to be heritably expressed under the control of a ubiquitous promoter (*actin*). Expression of *Gal4* transactivates the expression of *UAS-GFP*, thereby causing all progenitor cells and their progeny to inherit GFP expression. This system allows for monitoring of midgut renewal in varying conditions (Fig. 2b).

7. Under basal conditions, about 90 % of *Drosophila* ISC divisions occur asymmetrically, resulting in one daughter cell committed to differentiation and a second daughter cell that retains pluripotency [12, 13, 24]. The remaining 10 % are symmetric divisions, yielding either two differentiating cells or two ISCs, leading to the loss or expansion of stem cell clones in the gut. Twin-spot MARCM (Mosaic Analysis with a Repressible Cell Marker) allows labeling of the two daughter cells of an ISC with distinct fluorescent markers (GFP or RFP) (Fig. 2c). In the case of asymmetrical division, one daughter will differentiate and give rise to a single differentiated cell, whereas the other daughter will have ISC fate, and generate a clonal population. In the case of symmetrical division, either two single differentiated cells will be generated, or two ISCs that will generate two clones labeled in GFP and RFP.
8. Sucrose can be stored at 25 % concentration and at −20 °C in 1–2 mL aliquots.
9. **Steps 1–3** should always be performed using sterile techniques.
10. *Ecc15*, *P. entomophila*, and *S. marcescens* are grown at 29 °C; *P. aeruginosa* is grown at 37 °C.
11. Two hours of starvation at 29 °C are required to ensure that the flies will rapidly feed on the prepared sucrose and infection mix.
12. Flies infected by *Ecc15* are damaged in the first 4 h, which triggers ISC proliferation (massive from 8 to 16 h) and gut repair (up to 5 days).
13. Special care should be taken in each step to ensure that the midgut is never handled directly by the forceps. Pinching or even holding the guts with metal forceps will puncture or tear the tissue.
14. This removes cuticular hydrocarbons that will otherwise cause flies to float on PBS and not mix. This also further anesthetizes the flies.
15. The crop is often filled with bacteria prior to oral infection and this step is often necessary to prevent guts from floating during further processing, and to reduce the presence of free-floating bacteria during imaging.
16. Usual antibody dilutions in PBTA found in the literature: anti-GFP = 1:1,000, anti-RFP = 1:250, anti-PH3 = 1:1,000, anti-Prospero = 1:500,anti-PDM1 = 1:500,Alexa-anti-mouse = 1:500, Alexa-anti-rabbit = 1:500.

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References

1. Demerec M (1994) Biology of Drosophila. Cold Spring Harbor Laboratory Press, New York, NY
2. Buchon N, Broderick NA, Lemaitre B (2013) Gut homeostasis in a microbial world: insights from Drosophila melanogaster. Nat Rev Microbiol 11:615–626
3. Ohlstein B, Spradling A (2006) The adult Drosophila posterior midgut is maintained by pluripotent stem cells. Nature 439:470–474
4. Micchelli CA, Perrimon N (2006) Evidence that stem cells reside in the adult Drosophila midgut epithelium. Nature 439:475–479
5. Ohlstein B, Spradling A (2007) Multipotent Drosophila intestinal stem cells specify daughter cell fates by differential notch signaling. Science 315:988–992
6. Buchon N, Broderick NA, Poidevin M et al (2009) Drosophila intestinal response to bacterial infection: activation of host defense and stem cell proliferation. Cell Host Microbe 5:200–211
7. Jiang H, Patel PH, Kohlmaier A et al (2009) Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. Cell 137:1343–1355
8. Harrison DA, Perrimon N (1993) Simple and efficient generation of marked clones in Drosophila. Curr Biol 3:424–433
9. Lee T, Luo L (1999) Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22: 451–461
10. Wu JS, Luo L (2006) A protocol for mosaic analysis with a repressible cell marker (MARCM) in Drosophila. Nat Protoc 1:2583–2589
11. Singh SR, Mishra MK, Kango-Singh M et al (2012) Generation and staining of intestinal stem cell lineage in adult midgut. Methods Mol Biol 879:47–69
12. Yu HH, Chen CH, Shi L et al (2009) Twin-spot MARCM to reveal the developmental origin and identity of neurons. Nat Neurosci 12:947–953
13. de Navascués J, Perdigoto CN, Bian Y et al (2012) Drosophila midgut homeostasis involves neutral competition between symmetrically dividing intestinal stem cells. EMBO J 31: 2473–2485
14. Beebe K, Lee WC, Micchelli CA (2010) JAK/STAT signaling coordinates stem cell proliferation and multilineage differentiation in the Drosophila intestinal stem cell lineage. Dev Biol 338:28–37
15. Buchon N, Broderick NA, Chakrabarti S et al (2009) Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in Drosophila. Genes Dev 23:2333–2344
16. Vodovar N, Vinals M, Liehl P et al (2005) Drosophila host defense after oral infection by an entomopathogenic Pseudomonas species. Proc Natl Acad Sci U S A 102:11414–11419
17. Liehl P, Blight M, Vodovar N et al (2006) Prevalence of local immune response against oral infection in a Drosophila/Pseudomonas infection model. PLoS Pathog 2:e56
18. Opota O, Vallet-Gely I, Vincentelli R et al (2011) Monalysin, a novel ß-pore-forming toxin from the Drosophila pathogen Pseudomonas entomophila, contributes to host intestinal damage and lethality. PLoS Pathog 7:e1002259
19. Nehme NT, Liégeois S, Kele B et al (2007) A model of bacterial intestinal infections in Drosophila melanogaster. PLoS Pathog 3:e173
20. Cronin SJF, Nehme NT, Limmer S et al (2009) Genome-wide RNAi screen identifies genes involved in intestinal pathogenic bacterial infection. Science 325:340–343
21. Chatterjee M, Ip YT (2009) Pathogenic stimulation of intestinal stem cell response in Drosophila. J Cell Physiol 220:664–671
22. Apidianakis Y, Pitsouli C, Perrimon N et al (2009) Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. Proc Natl Acad Sci U S A 106:20883–20888
23. Zeng X, Chauhan C, Hou SX (2010) Characterization of midgut stem cell- and enteroblast-specific Gal4 lines in drosophila. Genesis 48:607–611
24. O'Brien LE, Soliman SS, Li X et al (2011) Altered modes of stem cell division drive adaptive intestinal growth. Cell 147:603–614